FORM PTO-1390 (Modified) (REV 11-98) U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY'S DOCKET NUMBER	U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR
PL-9813	To be assigned 09/674457

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/IB99/00907	7 May 1999	8 May 1998

TITLE OF INVENTION

Microfluidic Device

APPLICANT(S) FOR DO/EO/US

Anders Larsson, Klas Allmer, and Per Andersson

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☒ This is an express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).
4. ☒ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. ☒ A copy of the International Application as filed (35 U.S.C. 371 (c) (2))
   a. ☒ is transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☒ has been transmitted by the International Bureau.
   c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☐ A translation of the International Application into English (35 U.S.C. 371(c)(2)).
7. ☒ A copy of the International Search Report (PCT/ISA/210).
8. ☐ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371 (c)(3))
   a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☐ have been transmitted by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☐ have not been made and will not be made.
9. ☐ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
10. ☐ An oath or declaration of the inventor(s) (35 U.S.C. 371 (c)(4)).
11. ☐ A copy of the International Preliminary Examination Report (PCT/IPEA/409).
12. ☐ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371 (c)(5)).

Items 13 to 20 below concern document(s) or information included:

13. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
14. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
15. ☒ A **FIRST** preliminary amendment.
16. ☐ A **SECOND** or **SUBSEQUENT** preliminary amendment.
17. ☐ A substitute specification.
18. ☐ A change of power of attorney and/or address letter.
19. ☒ Certificate of Mailing by Express Mail
20. ☒ Other items or information:

Copy of the International Application as published by the International Bureau

Return Postcard

U.S. APPLICATION NO (IF KNOWN, SEE 37 CFR)	INTERNATIONAL APPLICATION NO.	ATTORNEY'S DOCKET NUMBER
09/674457 To be assigned	PCT/IB99/00907	PL-9813

21. The following fees are submitted:.

BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)) :

		CALCULATIONS PTO USE ONLY
☐ Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2) paid to USPTO and International Search Report not prepared by the EPO or JPO	$970.00	
☒ International preliminary examination fee (37 CFR 1.482) not paid to USPTO but Internation Search Report prepared by the EPO or JPO	$840.00	
☐ International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO	$690.00	
☐ International preliminary examination fee paid to USPTO (37 CFR 1.482) but all claims did not satisfy provisions of PCT Article 33(1)-(4)	$670.00	
☐ International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(1)-(4)	$96.00	
ENTER APPROPRIATE BASIC FEE AMOUNT =		$840.00
Surcharge of **$130.00** for furnishing the oath or declaration later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492 (e)).		$0.00

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE		
Total claims	19 - 20 =	0	x $18.00	$0.00	
Independent claims	1 - 3 =	0	x $78.00	$0.00	
Multiple Dependent Claims **(check if applicable)**.			☐	$0.00	
TOTAL OF ABOVE CALCULATIONS =				$840.00	
Reduction of 1/2 for filing by small entity, if applicable. Verified Small Entity Statement must also be filed (Note 37 CFR 1.9, 1.27, 1.28) **(check if applicable)**.			☐	$0.00	
SUBTOTAL =				$840.00	
Processing fee of **$130.00** for furnishing the English translation later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492 (f)).			+	$0.00	
TOTAL NATIONAL FEE =				$840.00	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31) **(check if applicable).**			☐	$0.00	
TOTAL FEES ENCLOSED =				$840.00	
				Amount to be: refunded	$
				charged	$

☐ A check in the amount of to cover the above fees is enclosed.

☒ Please charge my Deposit Account No. **500-588** in the amount of **$840.00** to cover the above fees.
A duplicate copy of this sheet is enclosed.

☒ The Commissioner is hereby authorized to charge any fees which may be required, or credit any overpayment to Deposit Account No. **500-588** A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

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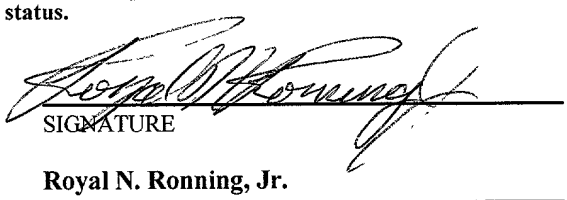
(732) 457-8423



SIGNATURE

Royal N. Ronning, Jr.
NAME

32,529
REGISTRATION NUMBER

October 30, 2000
DATE

PL-9813

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of:	A. Larsson, et al.	Group Art Unit:	To be assigned
Serial number:	To be assigned	Examiner:	To be assigned
Filed:	October 30, 2000		
For:	Microfluidic Device		

PRELIMINARY AMENDMENT

Honorable Assistant Commissioner of Patents
Washington, DC 20231

Sir:

In connection with the prosecution of the captioned application, which claims priority to an international patent application PCP/IB99/00907, filed May 7, 1999, please consider the following amendments and remarks.

IN THE CLAIMS

In Claim 3, line 1, please delete "either Claim 1 or Claim 2" and substitute -- Claim 1 – therefor.

In Claim 4, line 1, please delete "either Claim 2 or 3" and substitute - - Claim 2 - - therefor.

In Claim 6, line 1, please delete "Claims 4 or 5" and substitute - - Claim 4 - - therefor.

In Claim 9, line 1, please delete "either Claim 2 or 3" and substitute - - Claim 2 - - therefor.

In Claim 11, line 1, please delete "any one of Claims 4-10" and substitute - - Claim 4 - - therefor.

In Claim 17, line 1, please delete "any of the previous claims" and substitute - - Claim 1 - - therefor.

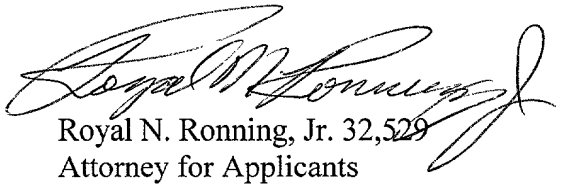
In Claim 19, line 1, please delete "either one of Claim 17 or 18" and substitute - - Claim 17 - - therefor.

REMARKS

Claims 1-19 are pending in the captioned application. Applicant has amended the Claims to delete multiple dependencies. Applicant respectfully asserts that such amendments are fairly based on the specification and respectfully request their entry.

Applicant believes that the claims, as amended, are in allowable form and earnestly solicit the allowance of Claims1-19.

Respectfully submitted,



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"MICROFLUIDIC DEVICE"

The present invention relates to microfluidic devices which may be used for a variety of biological processes, e.g. screening putative biologically active molecules against cell cultures or separating biological materials, the preparation of such devices and their use.

PCT patent application 97/21090 describes a microanalytical/ microsynthetic system for biological and chemical analysis which comprises a rotatable microplatform, for example a disk, having inlet ports, microchannels, detection chambers and outlet ports through which fluid may flow.

It has now been found that microfluidic devices can be prepared in which fluid flow may be controlled by having different surfaces of the substrate forming the device having different surface characteristics. By "microfluidic devices" is meant devices that can handle microvolumes of reagents, for example samples of less than 1μl, suitably less than 500nl and preferably between 1 and 10 nl, may be introduced into the device. By "fluid" is meant dry powders and liquids, including suspensions of particulates in liquids.

Accordingly, in a first aspect the present invention provides a microfluidic device adapted such that the flow of fluids within the device is controlled by different surfaces of the device having different surface characteristics.

The nature of the surface characteristics which control fluid flow is dependent upon the nature of the fluid itself. For example, when the fluid is a liquid, the surface characteristic that controls the flow of the liquid is preferably the surface energy of the material, e.g. low energy surfaces are normally hydrophobic whilst high energy surfaces are normally hydrophilic. The energy of a surface may be measured in terms of the critical surface tension(see for example Surface and Interfacial Aspects of Biomedical

Polymers, Vol I, Plenum Press, New York, 1985, Ch.7). When the fluid is particulate, the surface characteristic that controls the flow of the particles is dependent upon the nature of the particles, e.g. the surface is treated to interact with the particle, for example if the particle carries a charge the surface will have the same or opposite charge, similarly if the particle is magnetic the surface may be permanently or transiently magnetised.

In one embodiment there is provided a microfluidic device comprising a substrate whose surface is treated to provide areas having different surface characteristics, said areas being arranged to enable control of the flow of fluids passing across the substrate. For example, the substrate may have a hydrophobic surface interspersed with a plurality of hydrophilic areas. Alternatively, the substrate may have a hydrophilic surface interspersed with a plurality of hydrophobic areas. Preferably, the substrate is not formed from a hydrated oxide material. Preferably the substrate is formed from a plastics material such as a polycarbonate or a hydrocarbon polymer (including a halogenated hydrocarbon polymer) such as a polyolefin or a similar material which imparts a hydrophobic surface to the substrate. Whilst the substrate is formed from a material which provides a hydrophobic surface to the substrate, this hydrophobic surface can be treated, as described hereinafter, to convert it to a hydrophilic surface.

Preferably, the device has a second substrate approximately parallel to the first; the first, and optionally the second substrates having surface areas of different surface characteristics that control the flow of fluid within the device.

When the substrate comprises a hydrophobic surface interspersed with hydrophilic areas, these hydrophilic areas suitably comprise a plurality of arrays of hydrophilic spots on the hydrophobic surface. By an array of spots is meant a number of spots, suitably greater than 10 and preferably greater than 50, for example 200 , which are arranged on the surface

within the same fluid pathway in a predetermined pattern. The array may be single dimensional – i.e. a line of spots, or multi-dimensional.

By areas of different surface characteristics is meant that areas of the surfaces of the substrate have different relative characteristics, for example, in the case of liquids, different relative hydrophobicities or hydrophilicities. Boundaries between such areas may in effect form "walls" defining the flowpath of fluid within the device. Alternatively, they may form "valves" preventing the flow of fluid across the boundary until the fluid has either been provided with sufficient energy to enable it to overcome the difference in surface energies of the surfaces or, if the characteristic of the surface can be imparted to the surface transiently, e.g. in the form of an electric charge, magnetic field, particular temperature or light intensity, by changing the characteristic of the surface.

When a boundary between a hydrophilic and hydrophobic surface is used to create a valve, also referred to herein as a break, the physical parameters associated with the valve, or break, may be designed to give predetermined breakthrough pressures (that is to say the pressure required to make fluid pass over the boundary). Such physical parameters include the dimensions of the valve in terms of its width and breadth compared with the corresponding dimensions of the channel leading into it, the hydrophobicity of the surface forming the valve and, when the device is a rotational disk, the length of the channel leading into the valve.

Normally, it will be possible to pass fluid through a valve of the present invention a number of times. However, certain fluids (for example serum contains a high protein content) may modify the hydrophobic surface making this hydrophilic so that the valve only works once. In this case, when it is desired to add further fluid this will be introduced via a second channel, which also contains a hydrophobic/hydrophilic valve, which connects into the first channel.

It is believed that the terms hydrophobic and hydrophilic are well

known to those skilled in the art. That a surface is hydrophobic means that water does not spread on it but stands up in the form of droplets the contact angle being that measured from the plane of the surface, tangent to the water surface at the three phase boundary line. Thus, hydrophobic surfaces have been characterised as having high contact angles with water, often in the range 40 to 110 degrees (Zettlemeyer, Hydrophobic Surfaces, Ed.F.M.Fowkes, Academic Press, (New York). Hydrophilic surfaces are those which have low contact angles with water, often in the range 1 to 25 degrees. However, without limitation and for the purpose of guidance only, suitable hydrophobic surfaces include hydrocarbon polymers, including halogenated hydrocarbon polymers, see for example table 1, whilst suitable hydrophilic surfaces include non-contaminated metal oxides, silicaceous materials, such as glass and polysaccharides. Surfaces of materials may be modified to change their properties, i.e. hydrophilic materials may be given hydrophobic properties by surface treatment with a hydrophobic material such as hydrocarbon, perfluorinated hydrocarbon or silicone containing species. Likewise, hydrophobic materials can be made hydrophilic by the introduction of charged groups or hydroxyl, amide or polyether groups on the surface. It is often convenient to convert the whole (or substantially the whole) of a hydrophobic surface to a hydrophilic surface and to then introduce areas of hydrophobicity onto the hydrophilic surface. A small fraction of a monomolecular layer may be sufficient to change the surface characteristics drastically. When the hydrophobic/hydrophilic boundaries form "walls" and "valves", then the surface energy difference to form a wall may be the same or different to that for a valve, however the energy difference for a wall will normally be higher than that for a valve.

Some or all of the areas interspersed on the surface (be they hydrophobic or hydrophilic) may suitably be treated to allow the culture of cells on them. In this embodiment the device may for example be used for

screening intracellular events (see for example European Patent 650396 B on how this may be performed).

Suitable liquids for use in the devices of the present invention are those which have a surface tension preferably greater than 18mNm^{-1}. Aqueous solutions or suspensions which have a surface tension greater than 50mNm^{-1} are preferred.

Suitable particulates for use in the devices of the present invention are powders or beads having a particle size of less than 200μm. Such powders or beads are preferably treated in some way, for example they carry an electric charge or are magnetic, that makes them more amenable to flow through the device of the present invention. Whilst the present invention anticipates the use of particulates in the devices of the present invention in the absence of a liquid carrier, they may also be present in such a liquid carrier.

The microfluidic device is preferably circular and adapted for rotation about its axis. Such adaptation may take the form of a hole at the axis of one or both substrates which is capable of engaging a drive shaft. Other methods of rotating the device include clamping the device and contacting the perimeter with a moving surface, for example moving wheels, or placing the device on a turntable and spinning the turntable.

When the device is circular the fluid inlet is normally towards the axis of the device. The inlet may be a single port attached to an annular feed channel within the device or it may be a series of ports arranged at spaced angular intervals around the axis. An annular outlet is normally located towards the circumference of the device. Fluid may flow in a laminar manner across the surface of the device or it may flow in channels formed either by hydrophobic/hydrophilic boundaries or by interior walls connecting the two substrates. These interior walls are conveniently arranged radially around the axis of the device. The channels are normally of suitable dimensions to enable capillary forces to act upon the fluid within the channel.

When the device is adapted for cell culture it is preferable to have a source of gases available which aid cell growth. In this case, there will be one or more gas inlets in the device, which are conveniently situated in close proximity to the cells to be cultivated. Gas pathways are provided connecting the gas inlets to the cells or the fluid pathways connected to the cells, enabling culture medium/nutrients and gas, e.g. air, to be supplied down the fluid pathways.

The substrates forming the device are conveniently parallel and are preferably sufficiently close together to enable liquids in the device to be subject to capillary forces, suitably less than two millimetres apart, preferably less than one millimetre. Thus a liquid can be fed into the fluid inlet and will then be sucked down the fluid pathways by capillary action until it reaches a valve conveniently a hydrophobic/hydrophilic boundary, past which it cannot flow until further energy is applied. This energy may for example be provided by the centrifugal force created by rotating the device. Once the centrifugal force is sufficient, the liquid will flow over the valve and continue in an outward direction until it reaches the annular fluid outlet. When the areas interspersed on the surface are hydrophilic, the fluid will have a surface tension greater than $50mNm^{-1}$, for example aqueous solutions or suspensions, and when they are hydrophobic the fluid will be hydrophobic, e.g. non polar organic solvents. Thus, the fluid will be attracted to the areas/spots on the surface.

In one embodiment the areas form arrays of spots of hydrophobicities or hydrophilicities of a predetermined pattern. Such arrays can be used to build up deposits of materials to be analysed e.g. antibodies, oligonucleotides or a chemical library. For example, droplets of solvents containing the material to be analysed form on the surface, the solvent evaporates and the material is deposited.

In a second embodiment pathways are formed between parallel substrates. In this case surfaces forming the fluid pathways may

themselves have areas of alternating hydrophobicity and hydrophilicity forming arrays of spots as above. These alternating areas of hydrophobicity/hydrophilicity may be formed on the surface of one or both substrates, e.g. one surface may have alternating areas whilst the opposing surface does not.

Alternatively, the fluid pathways may contain a substance for separating chemical/biological materials, e.g. a gel for chromatography or electrophoresis or beads may be trapped in the pathways for carrying out assays; for example, scintillation proximity assays or cells can be trapped in the pathways through specific surface recognition.

Areas of hydrophobicity/hydrophilicity on a surface may be formed by methods well known to those skilled in the art, for example:

1) Masking and plasma treatment

This is applicable to most surfaces and enables different degrees of hydrophilicity/hydrophobicity to be achieved with ease. A mask (adhesive tape or cast film) is attached so that it fits tightly to all the surface features. Plasma treatment is then carried out on the non-masked surface.

2) Hydrophilic "photoresist"

The plastic surface is coated with a very thin layer of hydrophilic polymer (e.g. a polyIvinylcinnamate) which is crosslinked by illumination through a mask. Non-crosslinked polymer is washed off.

3) Crosslinkable surface active polymer

A surface active, reactive polymer is adsorbed from aqueous solution to the plastic surfaces and illuminated through a mask. Non-crosslinked polymer is washed off.

4) Polymerisable surfactants

A monolayer of polymerisable surfactant (e.g. the diacetylene functional phopholipids from Biocompatibles Ltd) is adsorbed and illuminated through a mask. Non-crosslinked surfactant is washed off.

5) Photo-oxidation

The plastic surfaces are illuminated with a powerful light source (e.g. Hg lamp or uv laser) through a mask so that the illuminated areas are oxidised by atmospheric oxygen.

6) Electron beam treatment

The plastic is irradiated through a mask so that irradiated areas are in contact with air (or other reactive medium) and are oxidised creating hydrophilic groups.

In order that the invention may be better understood, several embodiments thereof will now be described by way of example only and with reference to the accompanying drawings in which:-

Figure 1 is a diagram of a surface treated in accordance with the invention;

Figures 2 and 3 are diagrams similar to Figure 1, showing different arrangements;

Figure 4 is a diagram of a twin substrate microfluidic device according to the invention;

Figure 5 is a diagram to illustrate the use of hydrophilic areas to grow cells;

Figure 6 is a partial plan view of a rotary disc microfluidic device according to the invention; and

Figure 7 is a view of part of Figure 5, illustrated in greater detail.

Referring firstly to Figure 1, there is shown a mask with an array of 6x6 hydrophilic spots 1, each of 3x3 mm on a 50x50 mm hydrophobic surface 2, which was made in Mac DrawPro and printed on a laser printer. The printout was copied on to a transparency sheet in a copying machine.

The volume of a 25 mm thick film on a 50x50 mm surface 2 is 62.5 ml. This volume polyacrylamid (PAA) was deposited on the hydrophobic side of a Gelbondä film and the above mask was placed on top of the droplet. The area under the mask was wetted by capillary forces (a small portion of the solution did end up outside the mask). Photopolymerisation

through the mask was carried out for 3 minutes exposure time. The mask was removed and the surface was rinsed with water. A clear pattern was visible due to the selective wetting at the PAA surface.

Figure 2 illustrates a disc substrate 3 having a hydrophobic surface on which are formed eight 6x5 arrays of hydrophilic spots 1. Figure 3 illustrates a one-dimensional array of hydrophilic spots 1 on a hydrophobic surface 4. As will be explained, with a suitable force applied, a fluid can be caused to pass from spot to spot so that the structure forms a defined channel for fluid flow.

Figure 4 illustrates an arrangement comprising top and bottom plates 5,6 in the form of rotatable discs, having a common axis of rotation. The discs are illustrated far apart, for the purpose of clarity; in practice, the discs will be spaced apart by a distance defined by annular supporting walls 7 which distance will be suitable for the movement of liquid between the plates by capillary action.

The top disc 5 is provided with inlet holes 8 for supplying liquids to the interior. Lining up with these are corresponding areas 9 on the upper surface of the bottom disc 6, which are hydrophilic. Passing in an axial direction between the areas 9 is an elongate area 10, which is also hydrophilic. The remaining parts of the upper surface of disc 6 are hydrophobic. The elongate area 10 effectively forms a channel for liquid between the areas 9. The hydrophilic surface of area 10, bounded on both sides by the hydrophobic upper surface of disc 6 ensures that the liquid pathway is clearly defined by the "walls" which are formed by the interface between the hydrophobic and hydrophilic areas.

If the discs are rotated together about their common axis, it will be seen that centrifugal force will push liquid along the channel formed by area 10 from the innermost area 9 to the outermost area 9.

Figure 5 illustrates how cells might be applied to a hydrophilic area 2. An inlet 23 is provided for introduction of cells and reagent and a

hydrophobic channel 24 is provided for respiration of the cells during their growth on the area 2 and for rinsing between tests.

Reference is now made to Figures 6 and 7 which show a microfluidic device in the form of a compact disc (CD) 10 on which are formed hydrophobic and hydrophilic areas to enable liquids to be directed about the surface of the disc to enable the automatic and simultaneous carrying out of multiple chemical/biological tests on multiple samples.

Figure 6 shows a section of the compact disc 10, having a perimeter edge 11, and central hole 12 about which it may be mounted for rotation within a compact disc reader (not shown). On the surface of the compact disc are formed 40 sector-shaped multi-dimensional arrays 16 of hydrophilic spots. As is made clear in the enlarged view A in Figure 7, the spots are arranged in individual straight channels 13 radiating radially from the centre of the disc. Each channel comprises alternate hydrophobic areas or breaks 14 and hydrophilic areas or spots 15. The hydrophobic breaks 14 are typically 75 μm wide in the radial direction. The hydrophilic spots 15 are typically 108 μm wide in the radial direction.

In the illustrated embodiment, there are 20 channels in each array 16 and there are 200 hydrophilic spots 15 in each channel. Thus, each array 16 contains 4000 hydrophilic spots.

The channels in each array 16 begin in a common hydrophilic area 17 and end in a common hydrophobic area 18, constituting a break. Positioned radially outwards from the hydrophobic area 18 is a common waste channel 19.

Liquid reagent for use in carrying out the tests is introduced into an inner annular channel 20 which is common to all of the arrays 16. Extending from the channel 20 are 40 radially extending hydrophobic breaks 21, each extending to the hydrophilic area 17 of a respective array 16. A sample to be tested is introduced into the hydrophilic area 16 at 22. In this way, 40 different samples can be tested simultaneously.

Sample testing is carried out by applying to each of the hydrophilic areas 14 a sample of a known reactant, for example a known oligonucleotide. It will be seen that the device has the potential for testing each sample against 4000 different reactants. A cap may be formed on each hydrophilic spot by evaporation and accurate pre-concentration will occur on vaporisation.

Next the reagent channel 20 is filled and the disc is spun to cause the reagent to jump across the "valve" caused by the hydrophobic break 21 and radially outwardly to the waste channel 19. Progress along the individual channels 13 is by a series of jumps across the effective "valves" caused by the hydrophilic breaks 14. The force required to overcome the breaks is provided by the centrifugal action of the spinning disc.

Once the reagent is issuing into the waste channel 19 the disc is stopped and liquid sample added at 22. Typically the sample volume is 0.1 μl. The disc is now spun at 2 alternating speeds (for hybridisation mixing) whereupon the centrifugal force will move the liquid plug out along channels 13, and capillary action will move the liquid back up. Typically, the sample volume required for each spot 15 is 44 pl.

Reading of the test results is carried out by examining the individual spots 15 using a suitable reader. After the test is completed, the disc may be rinsed by the application of a suitable rinse liquid to the channel 20 and spinning of the disc to move the rinse liquid outwardly along channels 13 by centrifugal force.

Figure 8 shows a section of a CD, 23 having two consecutive inner annular hydrophilic channels, 24 and 25 which are connected by a radial hydrophilic channel 26 and a channel 27 which contains a hydrophobic area or break A. The outermost annular channel 25 is connected to an annular waste channel 28 by a radial hydrophilic overflow channel 29 having a hydrophobic break or valve Y2 adjacent to the junction with the

waste channel 28. The annular channel 25 is also connected to two serially arranged chambers 30 and 31, the second of which is in turn connected to the waste channel 28. The annular channels 25 and 28 and the chambers 30 and 31 are connected via channels which contain hydrophobic breaks or valves B,C and D.

The innermost chamber 30 has a treated surface permitting the growth of cells within the chamber. It is also provided with an air channel 32, which contains a hydrophobic break, and which, alternatively, can act as a sample inlet port. The outermost chamber 31 has an untreated hydrophilic surface and can conveniently act as an analysis zone in conjunction with a detector (not shown).

Aqueous reagent for use in carrying out tests is introduced into annular channel 25 and feeds by capillary action into the radial channels until it reaches the hydrophobic breaks or valves B and Y2. The CD is then spun at a first rotation speed so that liquid passes through Y2 into the waste channel 28 and then through B until it reaches C. Cells are allowed to grow in chamber 30 and when cell culture has reached the required level the disc is spun again at a second, higher rotation speed so that the contents of chamber 30 are transferred into chamber 31, but prevented from travelling further by the hydrophobic breaks or valves D. An analysis, or further manipulation, can then be carried out in chamber 31 after which the CD is spun at a third still higher, rotation speed so that the content of chamber 31 passes across D into the waste channel 28.

A rinse solution can then be introduced into the annular channel 24. The CD is spun again so that the solution passes through the hydrophilic breaks or valves Y and A, into the chambers 30 and 31 and then into the waste channel.

In order to prevent capillary "creep" of liquids around hydrophilic corners, a hydrophobic surface was applied to one side of the capillary channels, designated V in figure 8. (The channels are normally of square or rectangular cross section. The hydrophobicity and dimensions of the breaks or valves Y, Y2, A, B, C and D are chosen such that the force required to make liquid flow over D is greater than C which in turn is greater than B which is greater than Y2)

The following examples illustrate the preparation of surfaces having different characteristics on a hydrophobic substrate.

Example 1

A CD disc made from Zeonex (a cycloolefin copolymer manufactured by Nippon Zeon), having recessed microfabricated channels on the surface, was masked selectively by applying a viscous film-forming fluid at desired spots in the channels. As the film-forming fluid was used either Owoco Rod (based on a synthetic water-soluble polymer) or Owoco Rosa (based on a synthetic rubber latex dispersion), both delivered by Owoco AB, Stockholm, Sweden. After drying, the disc was placed in a plasma reactor (Plasma Science PS0500 from BOC Coating Technology, Concord Ca USA) and treated with an oxygen plasma (5 cm^3/min gas flow, 500 W RF power) for 10 min. The mask was then removed by water rinsing followed by an ethanol rinse. The non-masked areas had a water contact angle of 5 degrees, while the masked areas had a contact angle of 90 degrees. A soft silicone rubber lid was placed over the disc and an aqueous dye solution was introduced in the channels. The solution penetrated by self-suction into the non-masked channel areas, but stopped at the hydrophobic masked areas. By spinning the disc at 3000 rpm, the solution could be made to pass also over the masked areas.

Example 2

A CD disk made from polycarbonate, having recessed microfabricated channels on the surface, was placed in a plasma reactor (Plasma Science PS0500 from BOC Coating Technology, Concord Ca USA) and treated with an oxygen plasma (5 cm^3/min gas flow, 500 W RF power) for 10 min. After treatment the disc surface had a water contact angle of 5 degrees. A 0.5% solution of polyisobutylene in cyclohexane was then applied locally at selected spots and left to dry in. The polyisobutylene-coated areas had a water contact angle of 100 degrees. A soft silicone rubber lid was then placed over the disc and an aqueous dye solution was introduced in the channels. The solution penetrated by self-suction into the non-coated channel areas, but stopped at the hydrophobic coated areas. By spinning the disc at 3000 rpm, the solution could be made to pass also over the coated areas.

Example 3

A C.D. disk made from polycarbonate, having recessed microfabricated channels on the surface, was patterned with gold by evaporation through a shadow mask. First a 40nm think layer of chromium was evaporated through the mask. The CD disc was then placed in a plasma reactor (Plasma Science PS0500 from BOC Coating Technology, Concord Ca USA) and treated with an air plasma (10 cm^3/min gas flow, 500 W RF power) for 10 min. After treatment the disc surface had a water contact angle of 6 degrees. The CD disc was then placed in glass container and 50 ml of a 1 mM solution of octadecylmercaptane in ethanol was added. After one hour in the thiol solution, the CD disc was carefully rinsed by ethanol. The water contact angle on the polycarbonate area was 7 degrees, and 79 degrees on the gold surface. A soft silicone rubber lid was then placed over the disc and an aqueous dye solution was introduced

in the channels. The solution penetrated by self-suction into the non-coated channel areas, but stopped at the hydrophobic gold-coated areas. By spinning the disc at 3200 rpm, the solution could be made to pass also over the coated areas.

5

Table 1

Surface	Water contact angle (degrees)
Polytetrafluoro-ethylene (Teflon)*	108
Polyethylene*	94
Polypropylene*	95
Polymethyl methacrylate*	80
Platinum*	40
Glass**	"small"
Gold*	65.5

* A.C. Zettlemoyer (Hydrophobic surfaces, Ed P M Fowkes, Academic Press (New York) 1969, p.1-27

** A.W. Adamson Physical chemistry of surfaces 5th ed, Wiley-Interscience 1990, 9 397

CLAIMS

1. A microfluidic device adapted such that the flow of fluids within the device is controlled by different surfaces of the device having different surface characteristics.

2. A microfluidic device according to claim 1 comprising a substrate whose surface is treated to provide areas having different surface characteristics, said areas being arranged to enable control of the flow of fluids passing across the substrate.

3. A microfluidic device according to either claim 1 or 2 with the proviso that the substrate is not hydrated oxide material.

4. A microfluidic device according to either claim 2 or 3 wherein the substrate has a hydrophobic surface interspersed with hydrophilic areas.

5. A microfluidic device according to claim 4 further comprising a second substrate arranged approximately parallel to the first substrate such that fluid entering the device between the substrates will flow along predetermined pathways.

6. A device according to claims 4 or 5 wherein the plurality of hydrophilic areas is an array of hydrophilic spots.

7. A device according to claim 6 wherein the hydrophilic spots are arranged in lines radiating from a central point on the first substrate.

8. A device according to claim 7 wherein the lines of spots are separated by walls connecting the two substrates.

9. A microfluidic device according to either claim 2 or 3 wherein the substrate has hydrophobic and hydrophilic surface areas which define a pathway for fluid to travel over the surface in which there is at least one hydrophobic/ hydrophilic interface.

10. A microfluidic device according to claim 1, and having predetermined pathways for fluid flow, the surfaces of such pathways being hydrophilic, in which a valve is formed by a section in a pathway

having a hydrophobic surface.

11. A microfluidic device according to any one of claims 4 to 10 in which the surface of at least some of the hydrophilic surfaces is treated to enable the culture of cells.

12. A microfluidic device according to claim 11 which contains gas pathways to enable the access of air to the cell culture.

13. A microfluidic device according to claim 1 wherein the different surface characteristics are defined by different areas of the surface carrying different electrical charges.

14. A microfluidic device according to claim 13 wherein means are provided for changing the charge on the surface to alter the fluid pathway.

15. A microfluidic device according to claim 1 wherein the different surface characteristics are defined by different areas of the surface being differently magnetised.

16. A microfluidic device according to claim 15 wherein means are provided for changing the magnetisation of the surface to alter the fluid pathway.

17. A microfluidic device according to any of the previous claims which is circular.

18. A microfluidic device according to claim 17 which is adapted for rotation of the device.

19. A microfluidic device according to either one of claims 17 or 18 which has an inlet for fluids towards the centre of the device and an annular outlet for fluids towards the circumference of the device.

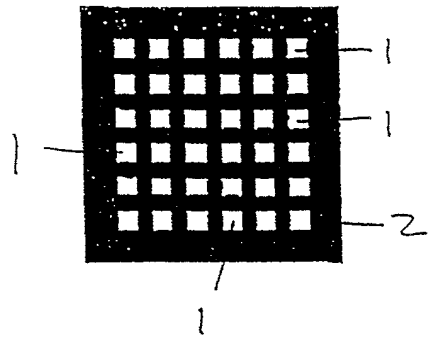

Figure 1

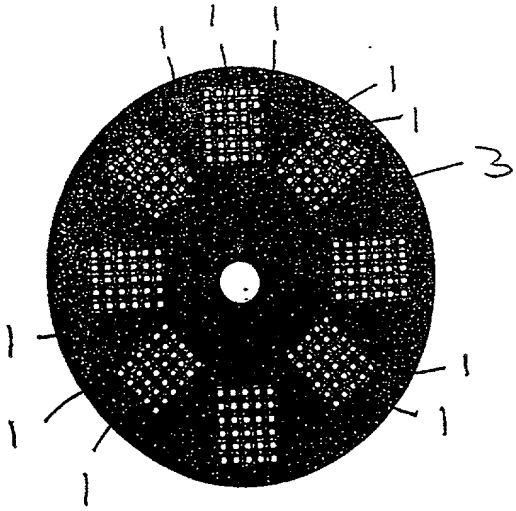

Figure 2

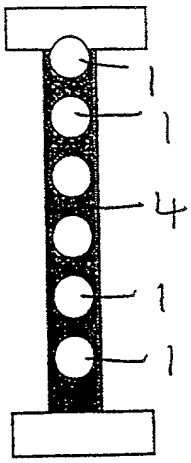

Figure 3

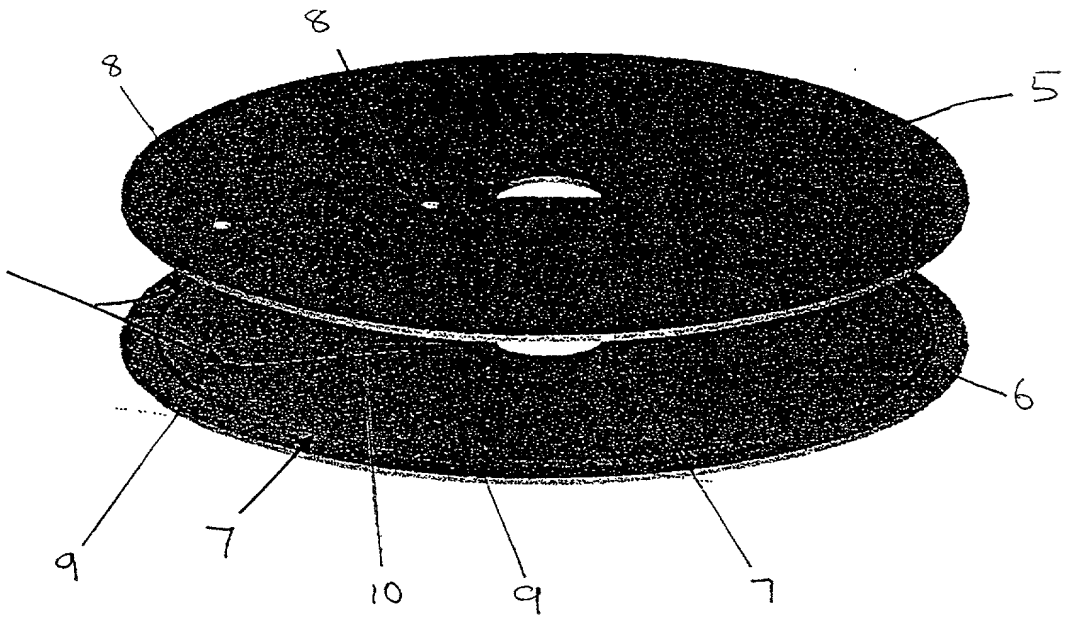

Figure 4

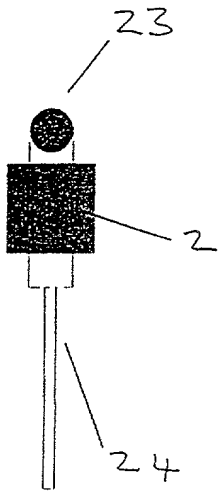

Figure 5

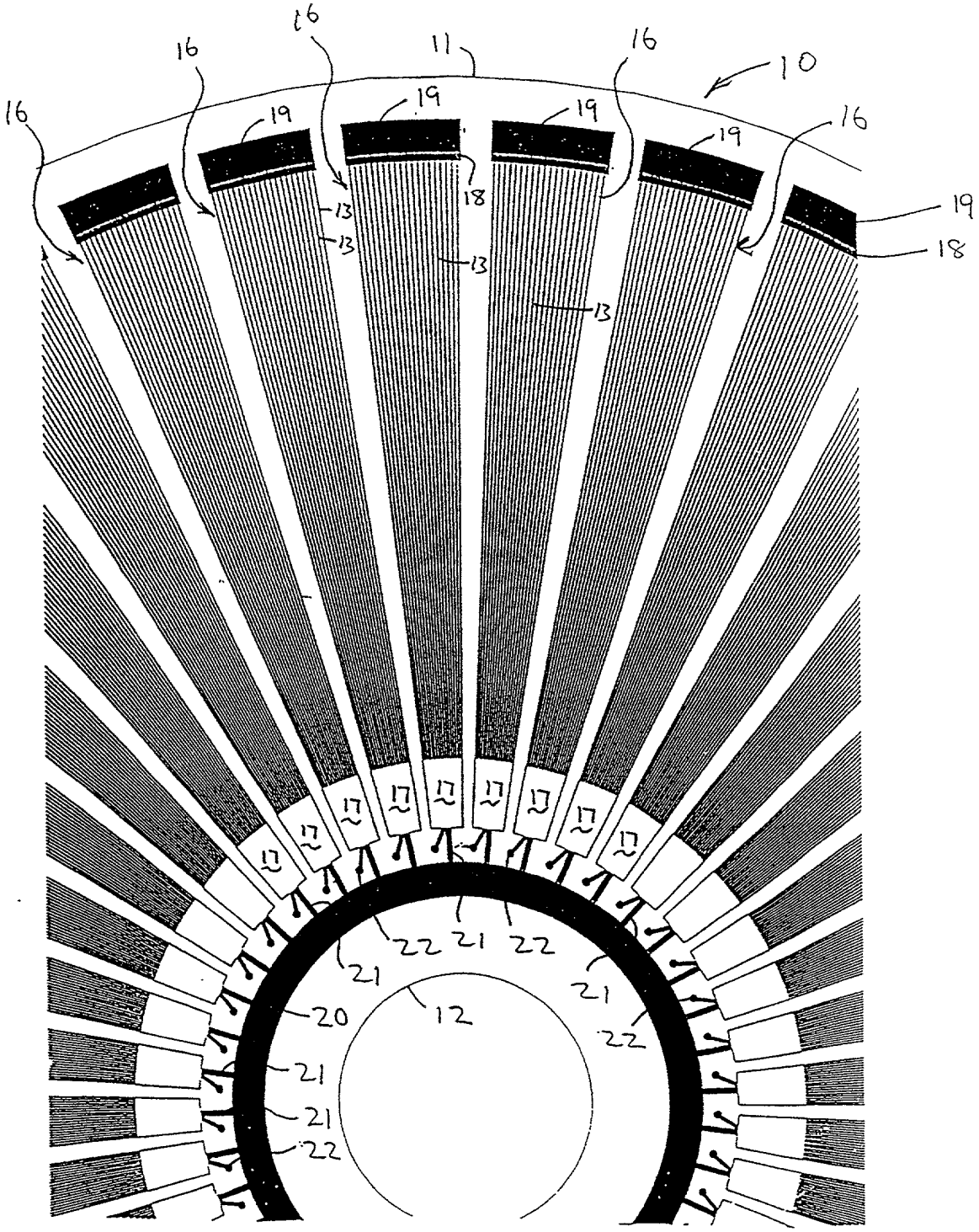

Figure 6

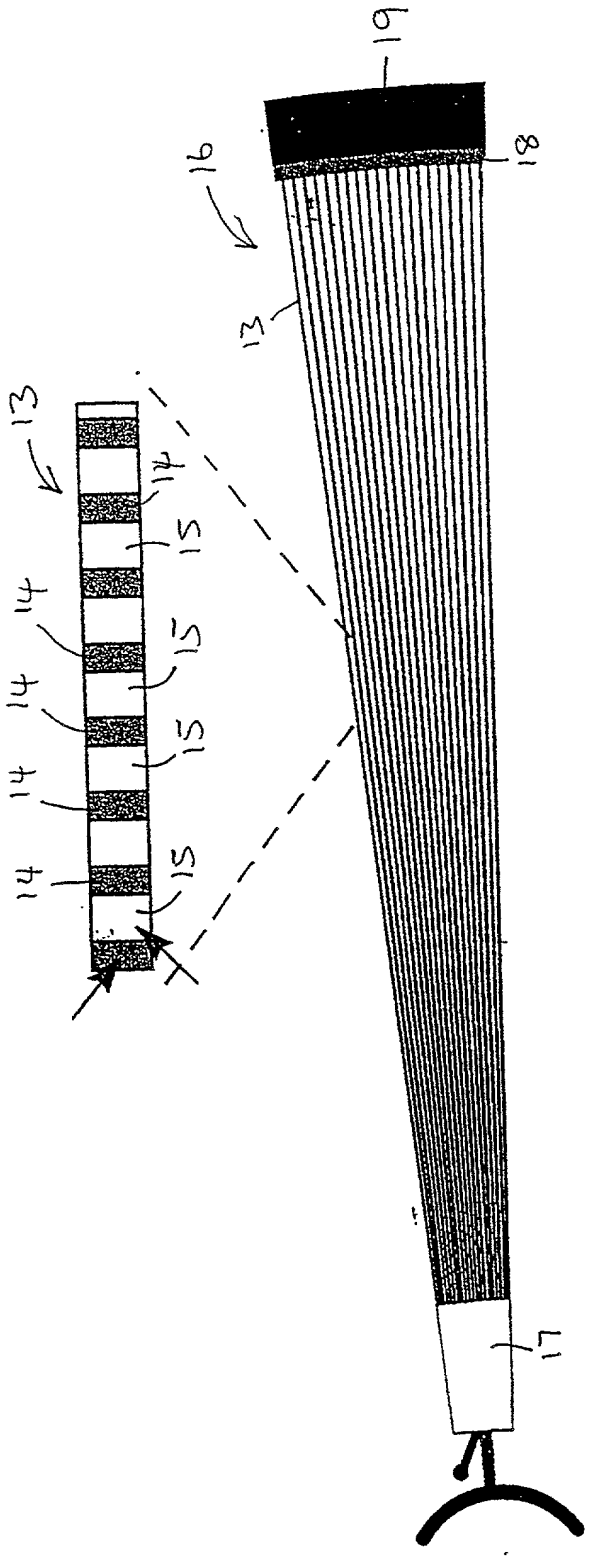

Figure 7

A-D Channel Width

Channel	A (µm)	B (µm)	C (µm)	D (µm)
	150	50	33	17

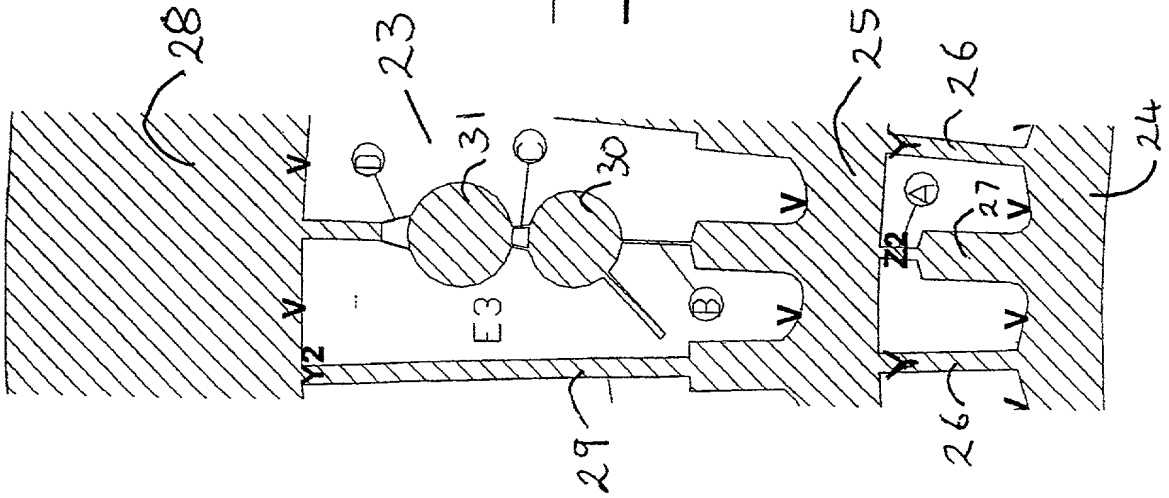

Figure 8

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DECLARATION FOR UTILITY OR DESIGN PATENT APPLICATION (37 CFR 1.63)

☐ Declaration Submitted with Initial Filing **OR** ☒ Declaration Submitted after Initial Filing (surcharge (37 CFR 1.16 (e)) required)

Attorney Docket Number	PL-9813
First Named Inventor	Larsson
COMPLETE IF KNOWN	
Application Number	09 / 674,457
Filing Date	30-Oct-2000
Group Art Unit	To be assigned
Examiner Name	To be assigned

As a below named inventor, I hereby declare that:

My residence, post office address, and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

Microfluidic Device

(Title of the Invention)

the specification of which

☐ is attached hereto

OR

☒ was filed on (MM/DD/YYYY) 10/30/2000 as United States Application Number or PCT International Application Number 09/674,457 and was amended on (MM/DD/YYYY) ______ (if applicable).

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment specifically referred to above.

I acknowledge the duty to disclose information which is material to patentability as defined in 37 CFR 1.56.

I hereby claim foreign priority benefits under 35 U.S.C. 119(a)-(d) or 365(b) of any foreign application(s) for patent or inventor's certificate, or 365(a) of any PCT international application which designated at least one country other than the United States of America, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate, or of any PCT international application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application Number(s)	Country	Foreign Filing Date (MM/DD/YYYY)	Priority Not Claimed	Certified Copy Attached? YES	NO
9809943.5	GB	05/08/1998	☐	☐	☒
			☐	☐	☐
			☐	☐	☐
			☐	☐	☐

☐ Additional foreign application numbers are listed on a supplemental priority data sheet PTO/SB/02B attached hereto:

I hereby claim the benefit under 35 U.S.C. 119(e) of any United States provisional application(s) listed below.

Application Number(s)	Filing Date (MM/DD/YYYY)	
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DECLARATION — Utility or Design Patent Application

I hereby claim the benefit under 35 U.S.C. 120 of any United States application(s), or 365(c) of any PCT international application designating the United States of America, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of 35 U.S.C. 112, I acknowledge the duty to disclose information which is material to patentability as defined in 37 CFR 1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application.

U.S. Parent Application or PCT Parent Number	Parent Filing Date (MM/DD/YYYY)	Parent Patent Number (if applicable)
PCT/IB99/00907	05/07/1999	

☐ Additional U.S. or PCT international application numbers are listed on a supplemental priority data sheet PTO/SB/02B attached hereto

As a named inventor, I hereby appoint the following registered practitioner(s) to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith: ☒ Customer Number 22840 OR ☐ Registered practitioner(s) name/registration number listed below → Place Customer Number Bar Code Label here 22840

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City		State		ZIP	
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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. 1001 and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

1-01

Name of Sole or First Inventor: ☐ A petition has been filed for this unsigned inventor

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☒ Additional inventors are being named on the 1 supplemental Additional Inventor(s) sheet(s) PTO/SB/02A attached hereto

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ADDITIONAL INVENTOR(S)
Supplemental Sheet
Page 1 of 1

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Residence: City		State		Country		Citizenship	
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Post Office Address							
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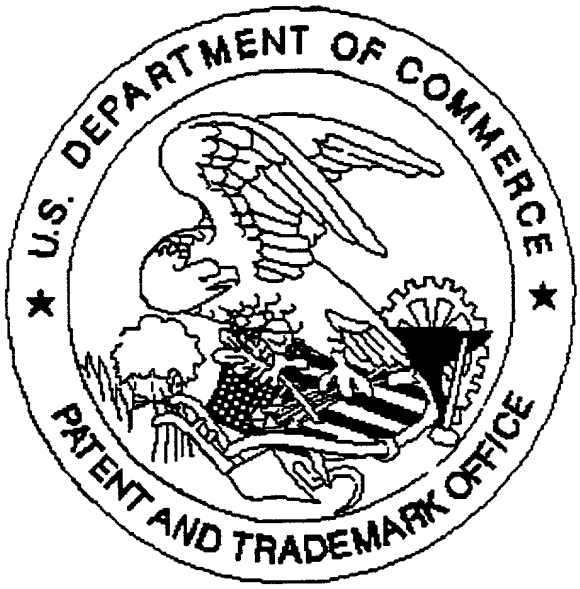

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